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Abstract

Advances in nanotechnology have the potential to lead to new techniques for developing silicon-based arrays for sensing biomarkers that may be associated with breast cancer. Until recently, breast cancer research has focused on a small number of genes or proteins as primary biomarkers. This approach has been extremely useful in clarifying general aspects of the disease. In order to develop patient-specific therapy, tailored for each individual, parallel detection of a large number ($\sim 10^3$-10^4) biomarkers may be required. There is a need for high throughput, sensitive methods for rapidly recording biomarker profiles of tumors in individual patients. We report results on the measurement of conductance change in bio-functionalized silicon nanowires. For large microscale sensors, the conductance is dominated by volume effects, which are not particularly sensitive to the binding of biomolecules to the surface. For nanoscale wires, such as those used in this study, the change is primarily due to the contribution of surface states to the conductance. The fractional change is greatest for the smallest sensors, due to the increased surface-to-volume ratio.

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INTRODUCTION

This is the annual report that describes the results of a research effort into the development of nanosensors for breast cancer biomarkers.

The proposal had three specific aims:

1. Design of a cantilever based sensor for biomolecular recognition, using Finite-Element simulation.
2. Fabrication of Biofunctionalized Nanoscale Sensors capable of detecting targeted molecules at a concentration of less than 1 ng/ml
3. Characterization of Functionalized nanosensors for selected breast cancer markers, and comparing with existing immunohistochemical and Fluorescence *in situ* hybridization (FISH) techniques on well established biomarkers such as Her-2/*neu*, estrogen and progesterone hormone receptors in tumor tissue, and selected mucin antigens in blood.

We are pleased to report that we have achieved two of the three specific aims and are now working on the last specific aim. During this year, we have discovered a very exciting gating principle using electrical methods that has the potential for significantly enhancing the sensitivity and control of the nanoscale structures. This gating idea, if confirmed can lead to cheaper and faster methods of detecting biomolecular markers. Combined with studies on tissue samples from patients, we feel that we will be able to meet all the specific aims of the IDEA award.

BODY OF REPORT

We report preliminary results on the sensitivity of nanoscale sensors by the measurement of conductance change of bio-functionalized nanowires. We have also performed simulation studies of the mechanical response of the sensors. Our results show that electrical measurements are very promising and provide a simple, effective and potentially inexpensive method for biomarker sensing. The change in conductance is primarily due to the contribution of surface states to the conductance, which for larger sensors is dominated by volume effects. The fractional change is greatest for the smallest sensors, due to the increased surface-to-volume ratio. Our silicon nanowires are fabricated from Silicon-On-Insulator wafer by electron beam lithography, which provides highly controllable nanowire sensors in comparison to other nanoelectronic approaches. We detect ultra-sensitive conductance change at nanoampere-level currents in functionalized nanowires with a silane-modified surface. The nanosensors are 200 nm wide and are among the smallest lithographically-controlled nanosensors that have fabricated for biomarker sensing.

Key Research Accomplishments

We list here the accomplishments so far:

Task 1: We have used simulations to characterize and develop nanoscale cantilever sensors. Simulations conducted in the presence of water show that hydrodynamic effects have to be taken into account in order to properly characterize the sensitivity of these sensors. At the

same time, we have performed electrical conductance measurements on nanonwires in order to assess the sensitivity.

Task 2: We have successfully used electron beam lithography to fabricate nanosensors and have functionalized them using silanization protocols. Some of the nanomechanical structures our collaboration has developed is among the smallest and highest frequency nanomechanical resonator ever constructed. In performing conductance measurements on the structures, we have discovered a very interesting gating effect that can be used to enhance the sensitivity of the nanosensors.

Task 3: Preliminary studies using a model system have been carried out and show great promise, and we propose to use the nanosensors for selected breast cancer biomarkers in the coming year.

Reportable Outcomes

We have used simulations to characterize and develop several types of nanosensors. Figure 1 shows a schematic of the electron-beam lithography method used to fabricate nanowire sensors attached to gold pads for electrical measurements.

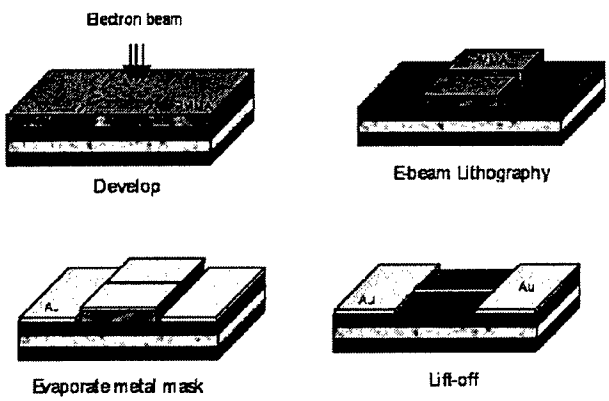


Figure 1. Schematic of electron-beam lithography used to fabricate a nanowire sensor in silicon. The gold pads are used for electrical measurements.

Figure 2 shows other structures that have been fabricated in the form of arrays of "tuning forks". At present, the structures shown represent the smallest and highest frequency nanomechanical resonators that anyone has ever fabricated for use in solutions.

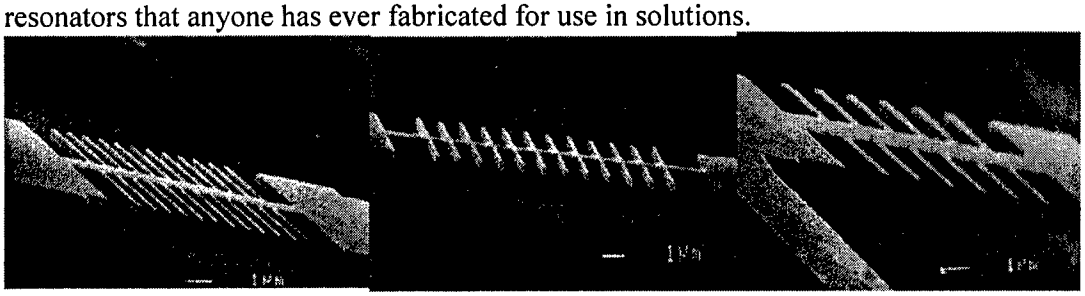
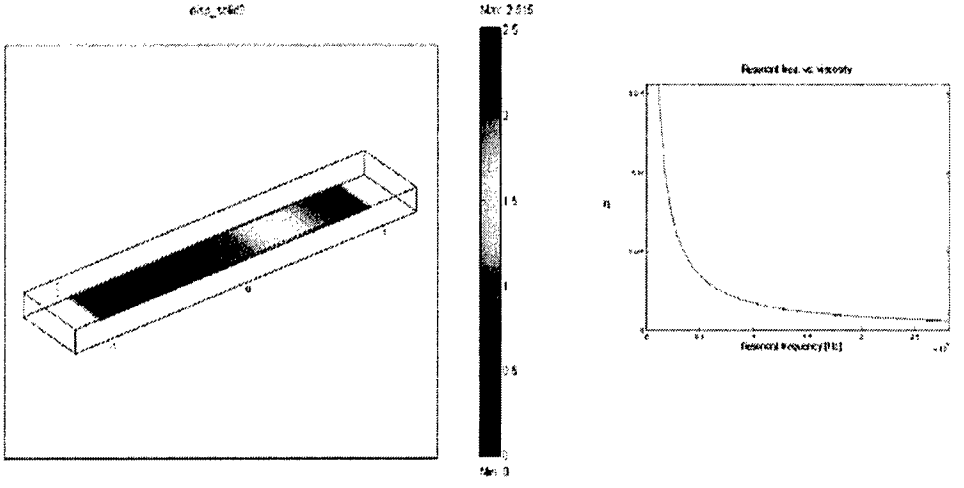


Figure 2. Electron microscope images of structures fabricated out of silicon. Note that the scale bar corresponds to 1 micron.

Figure 3 and Figure 4 show how the presence of hydrodynamic effects can change the nanomechanical properties of such nanoscale resonators.

Figure 3. Finite element simulations of the nanomechanical properties of the nanoscale tuning forks. The color indicates the distribution of the strain field in a the dominant normal mode.

When stress (load due to binding of biomarkers, for example) is applied, the response also depends on the hydrodynamic properties of the surrounding solvent. The effect of solvent viscosity is shown below in Figure 4.



Environment	Viscosity	Drag Force (Fd)	Max. Deflection y(L)	Resonant Frequency
Vacuum	0	0	0	2.18 MHz
Air	17.5 μkg/ms	26.2 μN	2.29 nm	~0.9 MHz
Water (blood)	1 mkg/ms	78.5 μN	6.88 nm	~63 kHz
Glycerol	1.5 kg/ms	39.3 mN	3.44 μm	~320 Hz

Figure 4. Effect of solvent viscosity on nanomechanical properties and the resonant frequency.

Simulations conducted in the presence of water show that hydrodynamic effects have to be taken into account in order to properly characterize the sensitivity of these sensors. We have also performed electrical conductance measurements on nanonwires in order to assess the sensitivity. Our results show that electrical conductance methods appear to be superior to mechanical methods for biomarker sensing, when the nanosensors become smaller than about 1 micron.

Shown in Figure 5 is an optical and electron microscope image of the biofunctionalized nanosensor used for electrical measurements. The engineering of our device consists of two fundamental steps: fabrication and functionalization. The silicon nanowire along with the side gates and the electrodes are fabricated by standard electron beam lithography and surface nanomachining. The starting SOI wafer has a device layer thickness of 230 nm and oxide layer thickness of 370 nm with a starting device-layer volume resistivity of 10-20 ohm-cm. The device-layer resistivity is further controlled by doping the wafer by ion implantation of boron with a concentration of $1x10^{18}$ /cc by ion implantation. After patterning the nanowires and the electrodes in separate steps with separate masks, the structure is etched out with an anisotropic reactive-ion etch (RIE).

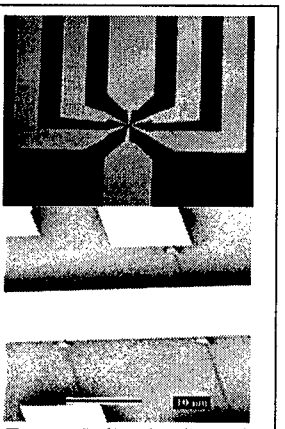


Figure 5: (*Top*) View of electrical pads connected to biofuncationalized nanosensor (*Bottom*)

Functionalization: The process described above exposes the three surfaces of the silicon nanowire along the longitudinal direction as shown in the schematic diagram in Figure 1(a). After the fabrication of the silicon nanowire and the gold electrodes and gates, a protective layer of polymethylmethacrylate (PMMA) is spun on the surface and only the silicon nanowire is exposed by a secondary *e*-beam exposure, while the device floor of oxide remains covered. This process allows exposure of only the silicon nanowire to air/solution. The functionalization of the nanowire surface is done by the application of a 2% APTES solution of methanol for 3 hours. After multiple rinsing of the device by methanol, the device is dried by nitrogen gas and baked at 80^{O}C in an oven for 10 minutes. fabrication method used. Characterization of surface functionalization was tested using a fluorescently labeled streptavidin-biotin system, and independently by an Atomic Force Microscope (Figure 6).

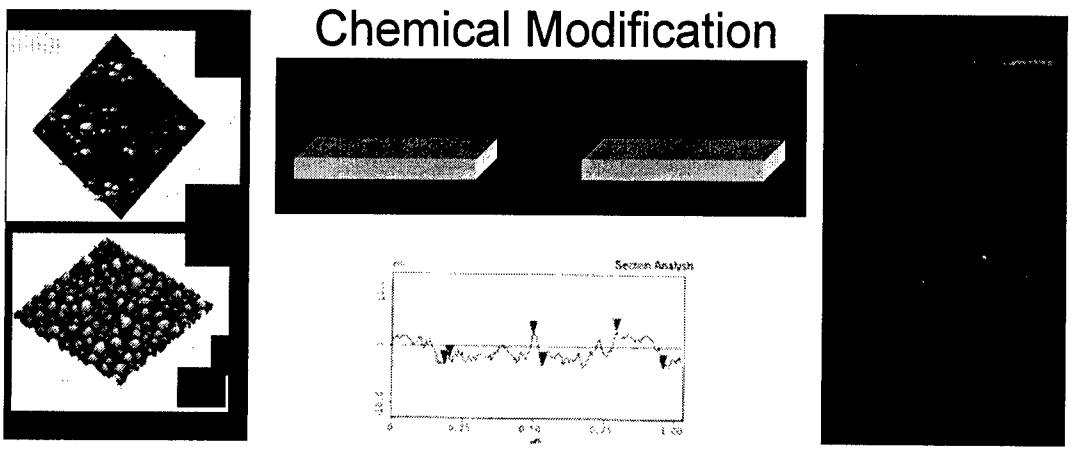


Figure 6. Silanization protocol and validation. (Left) Atomic Force microscope images of top of nanosensor, showing complete coverage. (Middle) Uniformity and Flatness test of the coverage shown in line scan on AFM. (Right) Fluorescence microscope images for validation of coverage using a fluorescent marker.

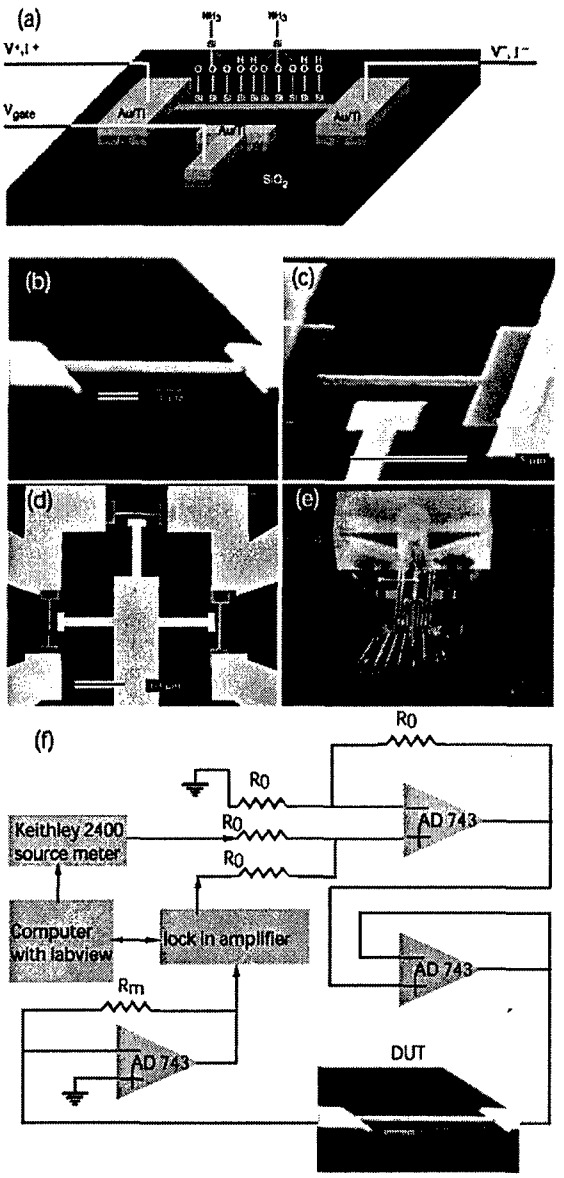


Figure 7. Device schematic diagram, scanning electron micrographs and measurement circuit. (a) The schematic diagram of the silicon nanowire with side gates and electrodes. The nanowire is exposed on three sides along the longitudinal directions. (b) The nanowire shown here is 300 nm wide, 230 nm thick and 8μm long. (c) A silicon nanowire with an Au/Ti side gate. (d) The scanning electron micrograph displays three silicon nanowire devices on the same chip. (e) An optical micrograph shows the flow chamber sealed on top of the devices on the interface board. (f) Schematic diagram of the differential conductance (*dI/dV*) measurement circuit shows the AC (EG&G 5210 lockin amplifier) and DC (Keithley 2400) sources. The circuit contains three AD 743 operational amplifiers, integrated with other elements on a PCB board. The entire circuit RF-shielded in an aluminum box. For the measurements, the circuit resistors have the following value: R_0=50 kΩ, R_m=100 kΩ.

Figure 7 shows the electrical schematic diagram of the electrical measurements used to characterize the functionalized nanosensors. Of particular interest in the location of a control gate in the devices in Fig 7 (c) and Fig 7 (d). This gate has allowed us to enhance the sensitivity and to control the labeling of the nanowire leading to a new method of using nanosensors for biomarkers. Without any gate biasing, we can measure the presence of the model marker at a

concentration of less than 1 ng/ml. By adjusting the gate voltage, the sensitivity is increased by atleast 1 order of magnitude.

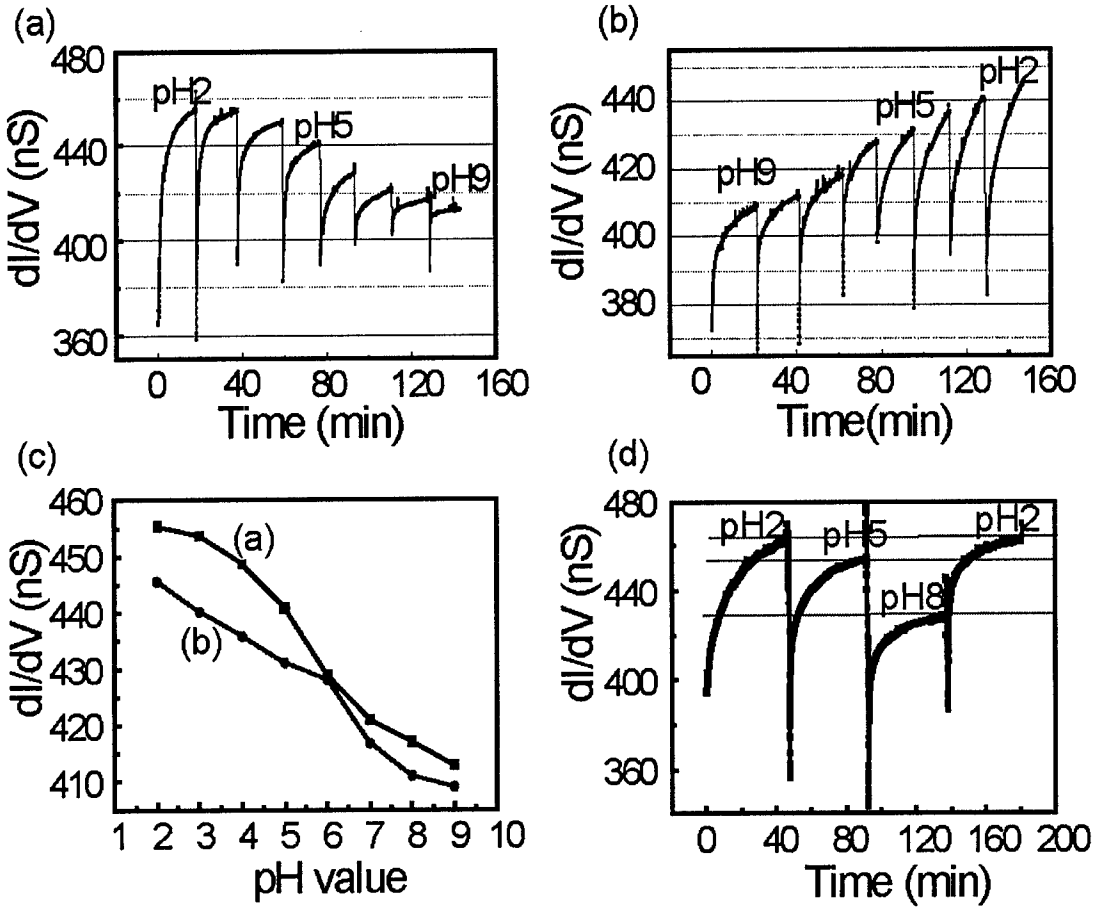


Figure 8. Zero-bias differential conductance (*dI/dV*) with increasing (a) and decreasing (b) pH values of the solution. (c) *dI/dV* for the two sets of measurements shown in (a) and (b). (d) *dI/dV* for an arbitrary sequence of pH values demonstrates reproducibility. The measurements are done with an AC drive ay 37 Hz with an amplitude of 50 mV.

As a test, the nanosensors were used to sense hydrogen ion concentration (pH, Figure 8). The estimated sample volume sensed is about 1 femtoliter. More interestingly, the effect of pH can be mimicked by changing the gate voltage, increasing sensitivity and control by electrically "tuning" the electric field near the nanosensor past the pK value of a selected biomarker target group. This ability of using gate voltage to increase sensitivity is shown in the last figure (Figure 9, next page). Increasing the gate voltage increases the differential conductance change as the ion concentration is changed. Conversely, the gate voltage can be used to "tune" the effective local ion concentration near the nanosensor, using a Field-Effect principle. This suggests that a suitable gate voltage can be used effectively to change "local pH" in a few femtoliters of solvent that are near the nanosensor. By changing the local proton ion concentration past the nominal chosen pK value, it is possible to control simply by using gate voltages the ability of a biomarker to bind or not, thus allowing for selective coating of the nanowire with specific antibodies or peptides. This aspect will be investigated using specific breast cancer biomarkers.

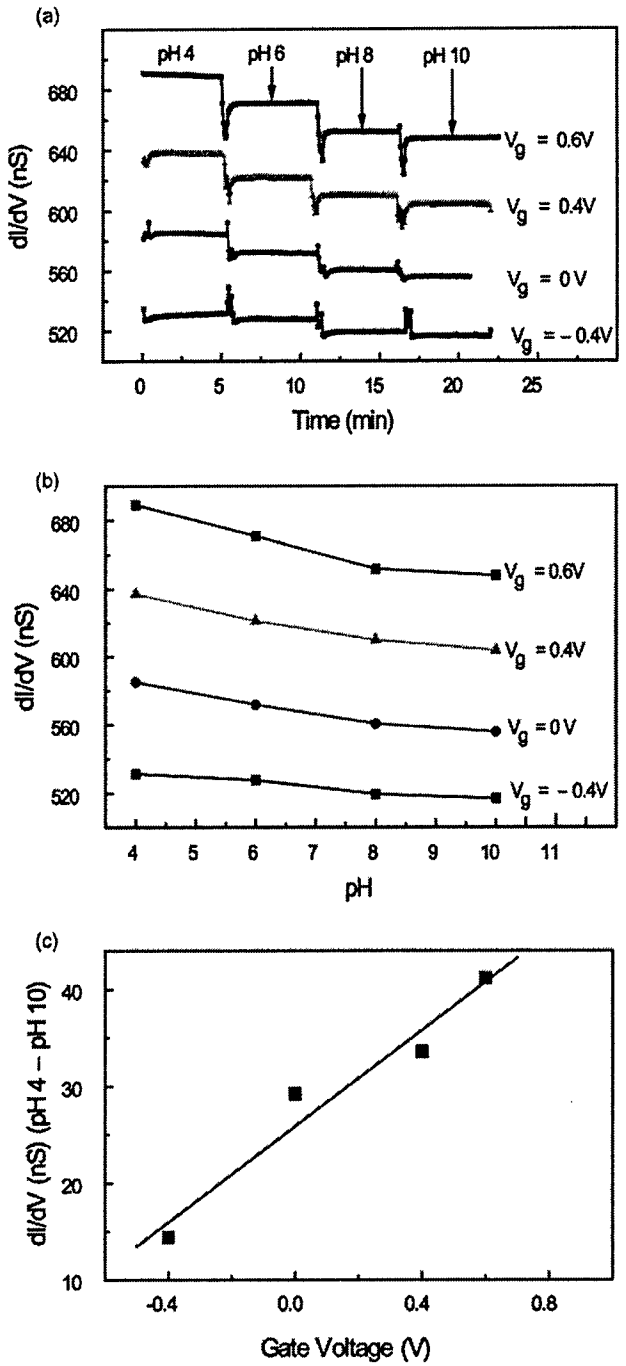


Figure 9. The Gate Voltage can be used to enhance sensitivity and control. Note that by changing the gate bias, the sensitivity can be increased (a).

Conclusions

To summarize our key research accomplishments, we have completed Task 1 and Task 2. We have discovered a potentially extremely interesting "Gate Control" method for enhancing signal and also for control of the local pH at the femtoliter level. This will allow for selective targeting of biomarkers by tuning the gate bias past the nominal "pK" value and to regulate binding. Use of the nanosensors in selected biomarkers (Task 3) remains the main goal of the project now. The success of Task 1 and Task 2 was described in a poster during the Era of Hope session, and a publication describing the results so far is being prepared for submission.